Genetically engineered mouse models of pancreatic cancer: unravelling tumour biology and progressing translational oncology

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Published Online First
30 August 2011

ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) remains a devastating disease despite tremendous scientific efforts. Numerous trials have failed to improve the outcome on this deadliest of all major cancers. Potential causes include a still insufficient understanding of key features of this cancer and imperfect preclinical models for identification of active agents and mechanisms of therapeutic responses and resistance. Modern genetically engineered mouse models of PDAC faithfully recapitulate the genetic and biological evolution of human PDAC, thereby providing a potentially powerful tool for addressing tumour biological issues as well as strategies for early detection and assessment of responses to therapeutic interventions. Here, the authors will discuss opportunities and challenges in the application of genetically engineered mouse models for translational approaches in pancreatic cancer and provide a non-exhaustive list of examples with already existing or future clinical relevance.

INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of death in the Western world despite its comparably low incidence, demonstrating the lack of efficient therapeutic strategies in this most deadly of all tumours. Despite significant improvements in diagnostic imaging modalities and improved surgical outcome, still <4% of the patients survive longer than 5 years. This devastating situation is due to metastatic disease at the time of diagnosis in most cases and the fact that this cancer is highly resistant to systemic therapies. Standard systemic treatment of unresectable or metastatic PDAC has, in principle, not changed in 15 years despite tremendous gains in molecular knowledge and an ever-rising arsenal of targets and drugs. In fact, the nearly complete failure of novel promising drugs in large clinical phase III trials has considerably dampened many hopes that this tumour type responds to any kind of treatment. Lately, however, other solid cancers such as colorectal, lung or mammary cancer have witnessed successful treatment approaches using chemotherapy and targeted therapies and patients can now be stratified to certain targeted therapies based on the molecular characteristics of the tumour. Thus, major emphasis may be placed on understanding tumour biology, microenvironment and interaction of key signalling pathways for developing rational combinatorial therapeutic approaches, identification of biomarkers and selection of eligible patient subgroups.

Identification of novel, clinically meaningful approaches heavily relies on the availability of preclinical models that (1) are recapitulating the morphological and molecular key features of the disease and (2) offer high predictive value for clinically useful diagnostic and therapeutic interventions. Traditionally, cell-culture-based assays and xenograft models with either subcutaneous or orthotopic transplantation of cancer cells in immunodeficient mice have been used for evaluation of novel agents with numerous studies reporting promising results. However, most models lack some if not most of the key features of PDAC, including intratumoural genetic heterogeneity, desmoplasia and spontaneous metastasis among others. Thus, it is not surprising that the predictive value of experimentally rather simple xenograft models is low to absent. In fact, virtually none of these approaches has led to improvement in clinical care of the patients with PDAC, which is a depressing conclusion given the high financial and personal investments and ever-present limited resources.

One approach circumventing many of the aforementioned problems aimed at directly implanting surgical specimen in immunodeficient mice. Key features of endogenous PDAC including the genetic alterations could be retained, and this approach has been demonstrated to be useful for screening purposes and drug and biomarker evaluation prior to clinical trials.[1 2] This approach circumvents many of the disadvantages of classical xenografts but does not allow studying early carcinogenesis and disease progression as well as inflammatory and immunological host–tumour interactions.

In recent years, identification of the morphological and molecular cornerstones of pancreatic carcinogenesis and advances in genetic engineering techniques have been driving forces in developing complex mouse models of PDAC, recapitulating many of the key aspects of the disease. The purpose

of this review is to provide an overview of the exciting developments in modelling human PDAC using genetically engineered mouse models (GEMMs). We will highlight recent attempts to use these preclinical model systems for (1) better understanding the tumour biology and carcinogenesis of PDAC, (2) identification of key signalling pathways for targeted treatment strategies and (3) utilisation in preclinical chemopreventive and therapeutic trials.

PDAC and precursor lesions

Overt pancreatic cancer has a tremendously high rate of genetic alterations and chromosomal instability, probably being a major cause for the intrinsic resistance of this cancer to any therapeutic approaches. Given these dismal features, earlier diagnosis and a deeper understanding of the key signalling drivers are pertinent goals to improve prognosis of patients with PDAC. By understanding the molecular circuitry of normal cells developing into highly malignant cancer cells, one may envision concepts of earlier diagnosis, identification of risk factors and early treatment or chemopreventive approaches.

Careful in-depth clinicopathological analysis has helped to identify and classify precursor lesions in pancreatic carcinogenesis (reviewed in Hezel *et al*[3]). Currently, three appreciated precursor lesions have been defined, namely, *p*ancreatic *i*ntraepithelial *n*eoplasia (PanIN) as the most common precursor lesion, *i*ntraductal *p*apillary *m*ucinous *n*eoplasms (IPMNs) and *m*ucinous *c*ystic *n*eoplasms (MCNs). PanIN lesions are divided into three stages (1–3) depending on the grade of architectural and nuclear atypia. As will be discussed below, all of these lesions can be recapitulated in GEMMs (figure 1).

In humans, PanIN stages have been shown to correlate with increasing rates of genetic alterations as discussed below and PanIN3 lesions represent a *carcinoma* in situ. IPMN and MCN are cystic lesions, which are less well characterised regarding their molecular alterations and the risk of malignant transformation. While MCNs are rare diseases, IPMNs are increasingly recognised in clinical medicine likely due to better diagnostic imaging modalities and awareness of clinicians.

The identification and classification of precursor lesions that give rise to invasive pancreatic cancer have enabled and accelerated the enormous progress in defining the accompanying genetic and molecular events during cancer development and progression.[3] One of the earliest somatic mutations occurs in the *KRAS* oncogene, resulting in a constitutively active KRAS protein with persistent downstream signalling. In fact, since almost all PDACs harbour activating *KRAS* mutations, this mutation is thought of as a gatekeeper for initiating the carcinogenic process. Additional acquired high-frequency genetic alterations during PanIN progression include inactivation of the tumour suppressor genes *INK4a/ARF*, *TP53* and *SMAD4*. Besides these driver genes, many additional genetic and key signalling pathway alterations occur in full-blown PDAC,[4] aggravating identification and efficacy of successful therapeutic interventions.

GEMM FOR PDAC DISEASE MODELLING

With the advent of sophisticated molecular technologies for generation of transgenic mouse models at hand, identification of the early genetic initiator alterations in pancreatic precursor lesions has led to the development of GEMMs recapitulating important aspects of human PDAC. Most of the GEMMs used today and reviewed here use recombinases (eg, bacteriophage-P1-derived Cre recombinase) to excise a DNA sequence flanked and therefore recognised by specific short repeats (so-called loxP sites). While there are numerous other systems, which can be used to activate or inactivate genes (reviewed in Cheon and Orsulic[5]), most of the relevant GEMMs currently used in translational oncology are Cre/loxP-based models.

A major breakthrough emerged from the development of GEMMs with conditional Cre/loxP-based activation of an endogenous mutant *Kras* allele in pancreatic progenitor cells by the Tuveson laboratory.[6] Here, a mutant $Kras^{LSL\text{-}G12D}$ knock-in allele, silenced by the insertion of a LoxP-flanked STOP element, was activated by Cre-recombinase-mediated excision of the STOP element (figure 2A). This strategy proved to be highly successful as these mice faithfully recapitulate human PDAC with PanIN lesion development and progression to invasive and metastatic PDAC with increasing age.

A major caveat using the described Cre/loxP approach is the timing and targeted cellular compartment in which mutant *Kras* is activated, hence the choice of the Cre expressing strain. Strains available for targeting pancreatic progenitor cells include *Pdx1-Cre* transgenic or $Ptf1a^{+/Cre}$ knock-in strains, both of which become activated during early pancreatic development. Thus, mutant $Kras^{G12D}$ is activated during embryogenesis, which probably does not reflect the acquisition of sporadic mutations in adult cells in humans. Additionally, since the transcription factor PDX1 is expressed in the developing foregut (stomach and duodenum) as well as in the epidermis,[7] tumour development may occur in extrapancreatic organs, potentially affecting pancreatic carcinogenesis and responses to therapeutic approaches as well as the life span of respective mice. PTF1a, on the other hand, is expressed in the nervous system including brain, spine and retina.[8] Nevertheless, the *Pdx1-Cre;* $Kras^{G12D}$ and $Ptf1a^{+/Cre}$;$Kras^{G12D}$ models recapitulate many central characteristics of human PDAC in an astonishing way with PanIN lesions progressing over time to invasive and metastatic PDAC (figure 1).

As in humans, PDAC developed at an advanced age of the mice, typically not before 12–15 months of age despite occurrence of early-grade PanIN lesions starting a few weeks after birth. This observation has two major implications: (1) as suggested from human studies, development of

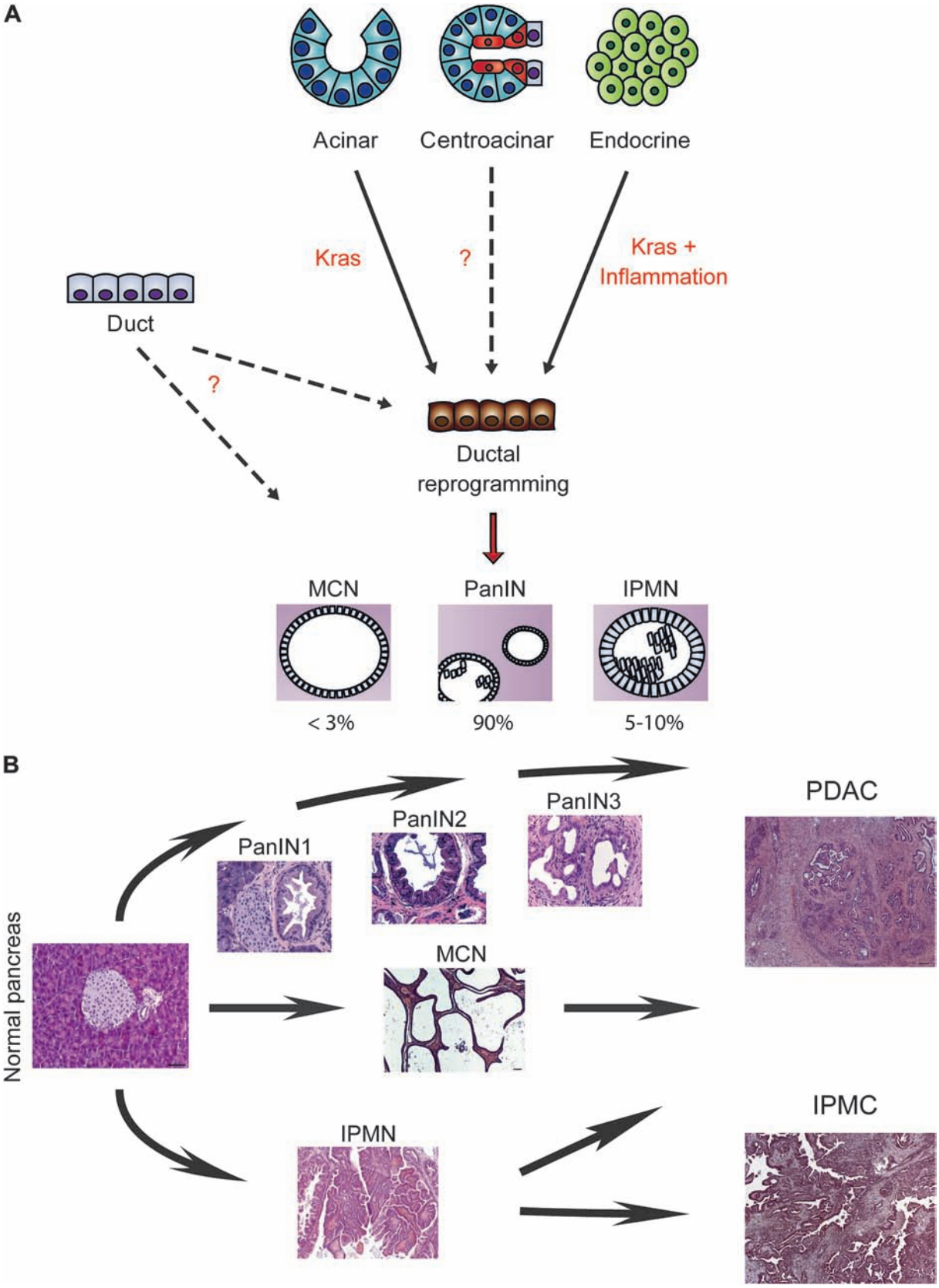

Figure 1 Routes to pancreatic ductal adenocarcinoma (PDAC) development. (A) Distinct pancreatic cell lineages can progress to different preneoplastic lesions by KRAS-induced ductal reprogramming. Different subtypes of non-invasive precursors of PDAC have been identified: microscopic pancreatic intraepithelial neoplasia (PanIN), intraductal papillary mucinous neoplasm (IPMN) and mucinous cystic neoplasm (MCN). (B) A classification system (grades 1—3) for PanINs, which is by far the most common precursor lesion, is based on morphological features including the degree of cell architecture abnormalities and nuclear atypia. Macroscopic (cystic) precursor lesions, IPMN and MCN, are cystic mucinous lesions, of which IPMNs can give rise to invasive IPMN [intraductal papillary mucinous carcinoma (IPMC)], whereas it is thought that all lesions can progress to invasive and metastatic PDAC.

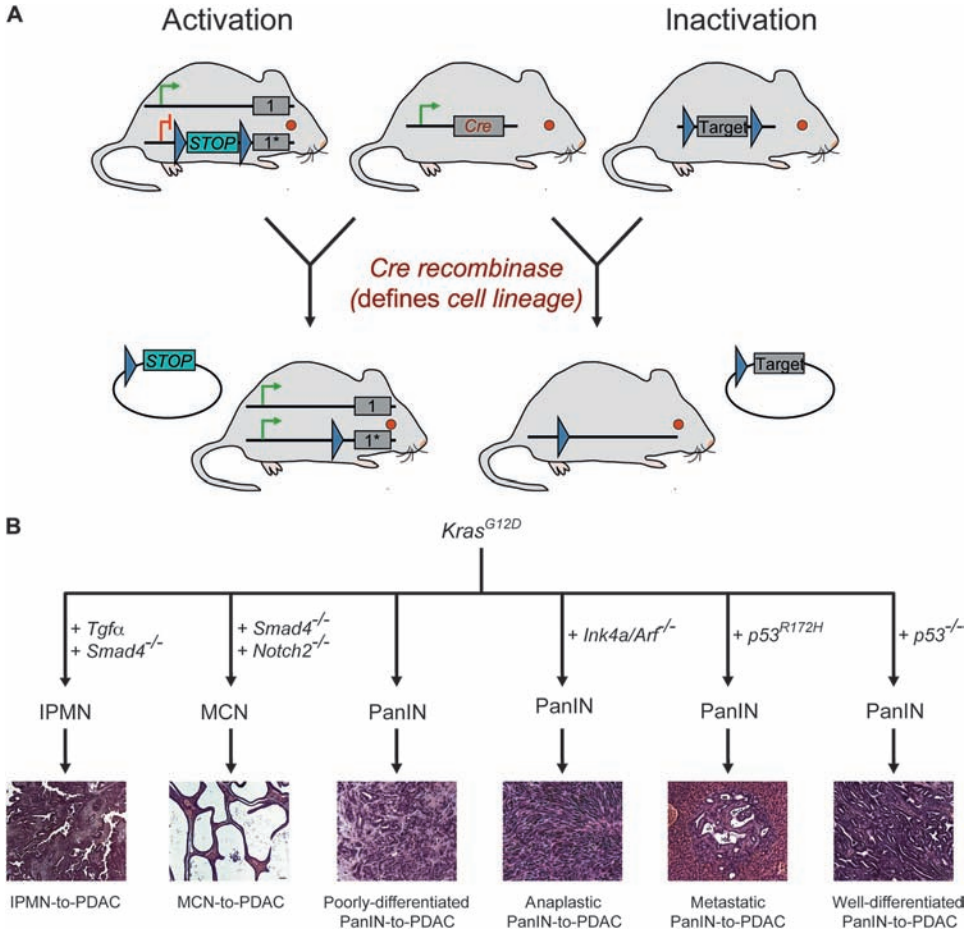

Figure 2 Mutant KRAS-driven genetically engineered mouse models (GEMMs) of pancreatic ductal adenocarcinoma (PDAC). (A) Cre/loxP-mediated conditional activation or inactivation of genes can be used for targeting oncogenes and tumour suppressors in the pancreas. (B) GEMMs develop tumours that resemble different types of human preneoplastic lesions and PDAC with varying latency depending on the induced genetic alterations and cancer evolution. IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; PanIN, pancreatic intraepithelial neoplasia.

full-blown PDAC requires further genetic alterations; (2) use of this model for translational therapeutic approaches is limited by the late and hardly predictable development of PDAC. A plethora of mouse models of pancreatic cancer has been developed by introducing additional genetic alterations. While the detailed characteristics of all of these models are beyond the scope of this review, we will focus on models that have an impact in understanding and treating PDAC. Table 1 gives a non-exhaustive overview of described GEMMs and their key characteristics.

ANALYSIS OF KEY SIGNALLING PATHWAYS

To study the role of genes that are thought to play a role for disease progression or that may be interesting targets, many research groups have used the widely available *Pdx1-Cre;Kras*^{G12D} or *Ptf1a*$^{+/Cre}$*; Kras*^{G12D} model. This model is characterised by a rather slow progression of PanIN lesions to invasive and metastatic PDAC and is thus well suited to study the impact of disease modifiers.

Given the loss of tumour suppressor genes *INK4a/ARF*, *P53* and *SMAD4*, conditional heterozygous or homozygous inactivation of these genes was a logical next step in studying PDAC using GEMMs. Conditional loss of the *Ink4a/Arf* locus in the *Pdx1-Cre;Kras*^{G12D} model led to acceleration of PanIN development, a greatly reduced tumour latency with an increase in undifferentiated and anaplastic PDAC, which showed micrometastasis to liver and lung.[11] Mice with conditional loss or dominant-negative mutations in the *p53* tumour suppressor gene (eg, *p53*^{R172H}) showed a highly accelerated development of PanINs and well-differentiated PDAC.[12 13] Interestingly, there seem to be some differences between biallelic conditional loss of *p53* and activation of a dominant-negative R172H mutation as mice with loss of *p53* do not show the metastatic phenotype of mice with mutant *p53*^{R172H}.[12 13] Several combined models with inactivation of *Smad4* showed a phenotype of cystic lesion development with similarity to human IPMN and MCN and may be valuable if tumour development and treatment are studied in this context.[15–17] Overall, targeting of key genes altered during pancreatic carcinogenesis leads to a variety of preneoplastic and PDAC phenotypes (figure 2B), demonstrating the cellular plasticity and distinctive functions of respective genes.

RAS, EPITHELIAL GROWTH FACTOR RECEPTOR (EGFR) AND EFFECTOR PATHWAYS

The central role of oncogenic KRAS signalling in pancreatic carcinogenesis makes it an attractive therapeutic target. However, successful inhibition of mutant KRAS remains an as of yet unmet goal. Reasons include the difficulties in developing drugs to target the intracellular GTPase, which is inhibited by classical *KRAS* mutations and thus

Table 1 GEMMs of PDAC

	Preneoplastic lesion			Cancer phenotype						
Genotype	**PanIN**	**IMPN**	**MCN**	**Onset (months)**	**Type**	**Grade**	**Metastasis (frequency)**	**Median survival (months)**	**Comments**	**Refs.**
Pdx1-Cre;Kras^{G12D}	Y			>12	PDAC	D	Y	>12	Long latency, spectrum of PanINs, Pdx1 expression in other organs7	6
Ptf1a$^{+/Cre}$;Kras^{G12D}	Y			>12	PDAC	D	Y	>12	Long latency, spectrum of PanINs	6
Ela-Tgfa	Y				Rarely cancer	NA	N	>12	Development of ADM and fibrosis, PDAC in p53 null background	9
Ptf1a$^{+/Cre}$;Kras^{G12D};Ela-Tgfa	Y	Y		5	PDAC	D	Y (50%)	7	PanIN and IMPN (pancreatobiliary subtype)-derived PDAC	28
Pdx1-Cre;Kras^{G12D};Ink4a/Arf$^{lox/lox}$	Y			2	PDAC	D/U	Y (11%)	2	PDAC with short latency and high penetrance, micrometastasis only	11
Pdx1-Cre;Kras^{G12D};Ink4a/Arf$^{+/-}$	Y			8	PDAC	D/U	Y (69%)	10	Longer latency than Ink4a/Arf-null mice, but gross metastasis	12
Pdx1-Cre;Kras^{G12D};Ink4a$^{-/-}$	Y				PDAC	U	Y (33%)	5	PDAC with short latency	12
Pdx1-Cre;Kras^{G12D};Ink4a$^{-/-}$;p53$^{lox/lox}$	Y			1.5	PDAC	U/D	Y (20%)	2	High penetrance and short latency	12
Pdx1-Cre;Kras^{G12D};p53$^{lox/lox}$	Y			1.5	PDAC	D	N	3	Well-differentiated PDAC with short latency	12
Pdx1-Cre;Kras^{G12D};p53$^{R172H/+}$	Y			2.5	PDAC	D	Y (63%)	5	Accelerated development of metastatic well-differentiated PDAC	13
Ptf1a$^{+/Cre}$;Kras^{G12D};Notch1$^{lox/lox}$	Y			>6	PDAC	D	Y (13%)	12	Similar or slightly accelerated PDAC development as Ptf1a$^{+/Cre}$;Kras^{G12D}	32 34
Ptf1a$^{+/Cre}$;Kras^{G12D};Notch2$^{lox/lox}$			Y	>9	PDAC	U	Y (50%)	>15	MCNs, only PanIN1, sarcomatoid PDAC with long latency	32
Pdx1-Cre;Kras^{G12D};Smad4$^{lox/lox}$		Y		4	PDAC	D	Y (37%)	9	Model of IPMN-to-PDAC progression	15 16
Ptf1a$^{+/Cre}$;Kras^{G12D};Smad4$^{lox/lox}$			Y	3.5	PDAC	D	Y (18%)	8	MCNs resembling human disease	17
Ptf1a$^{+/Cre}$;Kras^{G12D};p53$^{R270H/+}$;Brca2$^{Tr/D11}$	Y			2	ACC, PDAC	D	Y	2.5	Model of familial PDAC	60
Ela-tTA TRE-Cre;Kras^{G12V}	Y			12	PDAC	D	N	18	PDAC development after chronic pancreatitis	42
Ptf1a$^{+/Cre}$;Kras^{G12D};TGFβIIR$^{lox/lox}$	Y				PDAC	U	Y	2	Aggressive undifferentiated PDAC	20
Ela-CreERT;Kras^{G12D}	Y				No cancer	NA	N	>18	Acinar-derived PanIN development	14 29

ADM, acinar-ductal metaplasia; D, predominantly differentiated tumour; GEMM, genetically engineered mouse model; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; N, no; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; U, predominantly undifferentiated tumour; Y, yes.

needs to be pharmacologically enhanced and not inhibited. Other approaches such as the inhibition of the enzyme farnesyltransferase, which processes RAS proteins, showed no clinical effectiveness in PDAC.[23] While evidence for the initiating role of oncogenic KRAS in PDAC pathogenesis is fundamental, its function in tumour maintenance is not as clear. In a recent study, human PDAC could be classified into different molecular subtypes by global gene expression analysis.[24] These subtypes had differing responses to treatment with gemcitabine and erlotinib. The authors could identify one subtype showing KRAS dependency with better response to erlotinib while another subtype showing lower differentiation responded better to gemcitabine treatment. Of note, these subgroups were identified and defined in primary human samples but could also be identified in mouse cell lines derived from $Kras^{G12D}$-driven GEMMs with heterozygously floxed *Ink4a/Arf* and *p53* alleles supporting the validity of these models to address and test clinically relevant hypotheses. Consequently, these GEMMs may now be used to facilitate and evaluate subtype-specific therapeutic approaches.

Major RAS effector pathways include the BRAF/MEK/ERK and PI3K pathways. For both pathways, numerous inhibitors are currently under intensive investigation in clinical trials. $Kras^{G12D}$-driven GEMMs show activation of MEK/ERK signalling in PanINs and PDAC; however, so far, no genetic or pharmacological inhibition of this axis in GEMMs has been reported.

Activation of the PI3K signalling pathway with its major downstream effectors AKT, p70-S6K and RAC1 has also been demonstrated during early pancreatic carcinogenesis. Although mutations in the catalytic subunit of PI3K or inactivating mutations or loss of the PTEN tumour suppressor are rare in PDAC, several GEMMs provide evidence for an important role of this pathway in PDAC development. Stanger and colleagues described a GEMM with conditional loss of PTEN, in which acinar-ductal metaplasia formed probably from centroacinar cells, a compartment that is located between acinar and ductal cells and has been associated with progenitor cell properties.[25] Interestingly, these lesions showed malignant transformation to PDAC without abundant PanIN development, supporting the importance of PI3K signalling as therapeutic target. Another study with activation of AKT and concomitant lineage tracing showed a similar phenotype and described transdifferentiation of acinar and endocrine β-cells to a ductal cell type, supporting the view that PI3K/AKT signalling is important for cell fate decisions and plasticity in adult pancreatic cells.[26] It remains to be seen if this cellular function can be observed in fully malignant PDAC, but one can envision therapeutic strategies, in which PDAC with ductal differentiation can be reprogrammed to a therapeutically more susceptible phenotype.

An interesting RAS/PI3K target is RAC1, which was recently described as essential for preneoplastic lesion development in a genetic approach using $Ptf1a^{+/Cre}$;$Kras^{G12D}$ mice with genetic ablation of *Rac1*. Specifically, loss of RAC1 led to impaired development of acinar-ductal metaplasia due to impaired actin rearrangements.[27] This study demonstrates the importance of neoplasia-associated actin rearrangement during the initial carcinogenic stages, a process not yet well understood in PDAC development. Unfortunately, combined $Kras^{G12D}$ activation and *Rac1* ablation did not allow assessing the function of RAC1 in PDAC, a question that could be addressed by pharmacological approaches.

Another key signalling pathway in many human tumours including PDAC is the EGFR family, which consists of four transmembrane cell surface receptor tyrosine kinases and, together with their ligands (eg, epithelial growth factor and transforming growth factor (TGF)α, is overexpressed in 90% of PDAC.[10] In a GEMM with concomitant activation of $Kras^{G12D}$ and EGFR signalling, in which activation of EGFR was induced by acinar-specific overexpression of TGFα, dramatically accelerated development and progression of PanINs to PDAC were observed.[28] Interestingly, these mice additionally developed IPMN lesions that also progressed to invasive IPMC (intraductal papillary mucinous carcinoma; figure 2B).

One important consideration when interpreting the described models is that activation of mutant *Kras* and loss of the respective tumour suppressor gene occur simultaneously (figure 2). This scenario is different from human pancreatic carcinogenesis, where loss of tumour suppressors typically occurs after *KRAS* mutations and is a result of oncogenic pressure and interaction of various signalling pathways. Nevertheless and interestingly enough regarding further understanding of the disease, the described models show highly varying phenotypes with regard to the developing preneoplastic lesions, tumour differentiation and metastatic behaviour. This demonstrates the tremendous pancreatic cell plasticity given that in all models, Cre-mediated recombination occurred in a PDX1- or PTF1a-positive progenitor cell lineage during embryogenesis. This plasticity of pancreatic cells to develop distinct phenotypes may be due to specific signalling events taking place that favour a specific route to PDAC or development of these tumours from distinct cells of origin or both.

The question of different cells of origin and the ability of different cell lineages to develop preneoplastic lesions and PDAC were recently addressed by using strains expressing Cre under different promoters and, hence, in different cellular compartments. Carriere *et al* found PanIN formation in Nestin-expressing pancreatic progenitor cells after $Kras^{G12D}$ activation,[21] while Habbe and colleagues found that activation of mutant $Kras^{G12D}$ in adult mature acinar cells resulted in spontaneous induction of PanIN lesions.[29] By using strains with Cre expression from adult exocrine and endocrine cells, Gidekel and colleagues found both cellular compartments to give rise to PanIN/PDAC

development. In insulin-positive endocrine cells, however, an additional inflammatory stimulus to mutant $Kras^{G12D}$ was needed, which was generated by inducing acute pancreatitis.[30] These results are notable for two reasons. First, they highlight the central role of mutant *KRAS* as the key genetic alteration for initiation of the carcinogenic cascade. Second, they demonstrate that different pancreatic progenitor and mature cell compartments are capable of initiating the carcinogenic process demonstrating the plasticity of pancreatic cells (figure 1A). It will thus be an important future task to define and dissect the interaction of key signalling events and pathways and their aberrant activities for progression of preneoplastic lesions to PDAC. Pathways that have been shown to play a central role in pancreatic progenitor and exocrine plasticity include developmental pathways such as Notch and Hedgehog (Hh) signalling, all of which are targets of interest for intervention.

DEVELOPMENTAL PATHWAYS

Notch

Notch signalling is a key regulator during development and tissue renewal. In a context-dependent manner, Notch signalling plays a critical role in many processes including cell proliferation, cell death, cell fate decisions and differentiation. Not surprisingly, Notch pathway components are upregulated in numerous cancers including pancreatic cancer.[22 31] In GEMM of PDAC, modulation of Notch signalling significantly alters the carcinogenic process, making Notch an interesting therapeutic target. Expression of an active form of Notch1 (NIC) cooperating with oncogenic $Kras^{G12D}$ promotes PanIN formation,[14] whereas deficiency of *Notch2* but not *Notch1* attenuated PanIN development and strongly delayed the onset of disease.[32] In agreement with those findings is a chemoprevention study, where suppression of Notch activation was achieved using a γ-secretase inhibitor. Notch inhibition suppressed PanIN formation and PDAC development in *Pdx1-Cre;* $Kras^{G12D}$*;*$p53^{lox/+}$ mice.[33] Unfortunately, the lack of specificity and adverse effects of γ-secretase inhibitors may limit their use. Notably, we and others found that loss of *Notch1* did not inhibit tumourigenesis or even led to an acceleration of PanIN formation.[32 34] These studies emphasise the diverse outcome of seemingly highly similar Notch receptors, which depend on timing and/or context of Notch signalling. Thus, dissecting the contribution of different Notch pathway members and cautious use of targeted therapies will be crucial for clinical success of such approaches. As γ-secretase inhibitors, while being used in clinical trials, have many potential disadvantages including lack of distinction between individual Notch receptors, toxicity and cross-effects with other signalling pathways, other strategies using short peptides or specialised antibodies to specifically target Notch paralogues have been reported,[35 36] which can be evaluated in such preclinical models.

Hedgehog

Similar to Notch, Hh signalling mediates communication between adjacent cells. In the pancreas, Hh signalling seems to be especially important in mesenchymal cells, which hereby maintain an extensive mesenchymal–epithelial crosstalk essential for proper pancreatic development. In PDAC, Hh signalling was initially thought to act in an autocrine fashion and some studies found a survival benefit and less tumour development in GEMM treated with inhibitors against Smoothened (Smo), a central receptor for canonical Hh signalling.[37 38] Recent studies, however, indicate that PDAC belongs to a class of Hh-driven tumours that are resistant to ligand inhibition.[39] Instead, paracrine secretion of Hh ligands from tumour cells to induce tumour-promoting Hh target genes in the adjacent stroma and ligand-independent activation of Gli transcription factors downstream of Hh are potential mechanisms. Support for this mechanism comes from a GEMM, in which activity of the pathway was found in the tumour stroma and activation of an oncogenic *Smo* allele in epithelial cells had no impact on pancreatic neoplasia.[40] Furthermore, deficiency of *Smo* did not influence PanIN/PDAC development. This study further showed that in PDAC, Gli activation is decoupled from upstream signalling and is regulated by TGFβ and KRAS.[41] These studies represent fine examples of how the usage of GEMMs facilitates hypothesis-driven approaches to answer clinically relevant questions. Studies using inhibitors of the Hh signalling pathway indeed found remodelling of the tumour stroma as will be described below in further detail.

INFLAMMATION AND ASSOCIATED PATHWAYS

Chronic pancreatitis and hereditary pancreatitis are risk factors for PDAC development.[19] Recently, several reports using GEMMs have linked inflammation to PDAC development. In a landmark study, Guerra and colleagues found accelerated PanIN and PDAC development in $Kras^{G12D}$-driven GEMMs treated with pancreatitis-inducing cerulein.[42] Importantly, this study showed that PanIN initiation can occur from differentiated acinar cells under concomitant chronic injury. This acinar origin of PanIN initiation was also described in other studies with acceleration of PanIN development with additional injury stress.[29 43] In addition to facilitating susceptibility to differentiated pancreatic cells, induction of inflammation also accelerates the progression of PanIN lesions to PDAC.[43 44] These studies support the view that induction of reprogramming by inflammatory signals increases the susceptibility of pancreatic cells to $Kras^{G12D}$-driven neoplastic transformation. This finding has also been made for differentiated endocrine cells[30] but, so far, not for ductal cells, long thought to be the cells of origin for PDAC.

An interesting target often found in an inflammatory setting is cyclooxygenase-2 (COX-2). COX-2 is expressed in 90% of human PDAC but undetected in normal tissue, suggesting a role in tumour

development.[45] Numerous studies on pancreatic cancer cell lines implicated COX-2 inhibition with reduced proliferation. In human and mouse PanINs, the level of COX-2 expression rises with higher severity of the histological abnormality.[6 46] Using a chemopreventive approach in a $Kras^{G12D}$-driven GEMM, treatment with the non-steroidal anti-inflammatory drug nimesulide that inhibits COX-2 led to reduced PanINs formation.[47] A similar GEMM was also used in a successful preclinical trial of the selective COX-2 inhibitor celecoxib that was given in combination with gemcitabine and MUC1-based vaccine. Treatment led to a complete lack of development of invasive disease and significant suppression of higher-grade PanIN development, supporting COX-2 as a cancer chemopreventive target in pancreatic cancer.[48]

Tumour-associated inflammation has been observed in many cancers. Antigen expression of tumour cells as a result of aberrant gene expression may attract cells from the immune system. However, interaction of cancer and immune cells has been reported to have tumour-suppressive and tumour-promoting roles dependent on the predominant leucocyte infiltration. In PDAC, leucocytes typically orchestrate an immunosuppressive immune reaction, which was also observed in $Kras^{G12D}$-driven GEMMs. Interestingly, leucocyte infiltration was already observed around early PanIN lesions persisting in desmoplasia of invasive PDAC.[49] In an attempt to reverse the immunosuppressive reaction, Beatty and colleagues treated chemotherapy-naïve patients with advanced PDAC with gemcitabine and an activating CD40 antibody.[50] Unexpectedly, activation of CD40 led to tumour regression requiring macrophages but not T cells. Samples taken from these patients surprisingly showed a predominant macrophage infiltration. Interestingly, when a GEMM cohort was treated, about a third showed tumour regression, and this effect was also dependent upon the presence of macrophages but not T cells. Thus, this study is a prime example of a translational study combining a clinical trial with a GEMM-driven experimental approach with the surprising findings observed in the patients being experimentally addressed in GEMM.

Further evidence for a key role of tumour-associated inflammation in PDAC has recently been demonstrated in two studies, which showed STAT3 activation to be required for PanIN initiation and progression.[51 52] One central observation besides regulation of proliferation and apoptosis by STAT3 was its role in controlling the microenvironment by producing cytokines and chemokines, thereby attracting inflammatory cells. These inflammatory cells further facilitated STAT3 activation through production of various cytokines including interleukin-6. Thus, disruption or blockade of inflammatory signals may be a feasible chemopreventive and possibly therapeutic approach in PDAC. A recent approach using triterpenoids and rexinoids alone and in combination showed strong efficacy in EGFR and STAT3 binding, leading to greatly improved survival in $Pdx1\text{-}Cre;Kras^{G12D};p53^{lox/+}$ mice.[53]

TRANSLATIONAL STUDIES

Besides the use of GEMM for basic aspects of pancreatic carcinogenesis, they are well suited for diagnostic studies such as biomarker identification, early detection and response evaluation as well as chemopreventive and therapeutic interventional studies. Based on the specific aim, selection of models can greatly vary and the following criteria should be taken into consideration: (1) histopathology: common with given human cancer pathological features; (2) natural history/tumour evolution: model should recapitulate disease progression as it occurs in humans—for example, local invasiveness and metastasis to similar sites as in human cancers; (3) origin: the ideal model will produce subtle, controlled mutations in relevant endogenous genes in targeted cells, while leaving an effectively wild-type genotype in non-targeted cells; (4) microenvironment: model should recapitulate contributions of microenvironment—for example, tumour stroma and immune system; (5) molecular pathways: oncogenes or knock-out genes that drive the model should mimic changes that are observed in the human disease; (6) environment: similar hormonal, dietary or other factors that affect disease progression in human should be relevant to the mouse models; (7) predictive utility of a model involves similar responses to preventive agents and drugs that have previously been tested in human patients. While GEMMs have been shown to indeed address many of these criteria to a certain extent, their predictive value regarding clinical relevance has yet to be demonstrated. In the next section, we will provide few recent examples of such GEMM studies.

DIAGNOSTIC APPROACHES: BIOMARKERS AND EARLY DETECTION

Utilisation of GEMMs for novel diagnostic approaches is a rapidly growing field in cancer research. Non-invasive assessment of the pancreas in GEMMs permits dynamic studies of tumour development and progression and allows evaluation of various imaging modalities such as CT, MRI, positron emission tomography (PET), ultrasound and optical imaging technologies that detect fluorescence and luminescence. Functional imaging modalities especially are promising tools for early disease detection and for differentiation between chronic inflammatory and malignant processes. While a detailed review of these approaches is beyond the focus of this article, few examples highlight the potential of this approach.

PET imaging has been widely used in clinical oncology for detection of small tumour sites, and 18F-fluorodeoxyglucose (FDG)-PET has been evaluated in PDAC imaging approaches.[54] In an Ela1-myc mouse model, which develops tumours of mixed acinar-ductal phenotype, FDG-PET detected tumours in an early state.[55] However, these mice do

not develop typical preneoplastic lesions and PDAC. Very recently, Fendrich and colleagues evaluated FDG-PET in *Pdx1-Cre;Kras*^{G12D} and *Pdx1-Cre;Kras*^{G12D}*;p53*^{R172H} mice for detection of PanIN lesions and PDAC, respectively.[56] Indeed, the authors reported a strong FDG uptake in PDAC and a weak signal in the pancreatic region of mice with PanIN lesions. These findings were associated with an elevated glucose metabolism in PanINs and, to a higher extent, in PDAC, supporting further exploration of PET imaging.

In an attempt to identify and validate novel methods of early detection of preneoplastic lesions, Eser and coworkers used a cathepsin-activatable near-infrared probe in combination with flexible confocal laser microscopy for detection and grading of PanIN lesions in a GEMM of PDAC.[57] This novel endoscopic technique was found to be very sensitive and specific for detection of PanINs and may thus be very interesting for translation into the clinic—for example, screening patients at high risk for developing PDAC.

Olive and Tuveson used small-animal high-resolution ultrasound to detect and measure the volume of endogenous PDAC as small as 1 mm, a prerequisite for choosing the right time point for starting therapeutic interventions.[58] By using contrast ultrasound and dynamic contrast-enhanced MRI, they hypothesised decreased tumour perfusion and, thus, poor drug delivery as potential mechanisms for therapeutic resistance of PDAC.[38]

Besides diagnostic imaging, identification of novel biomarkers would be helpful for early detection of PDAC. A proteomic approach analysing the plasma proteome of a GEMM driven by oncogenic *Kras*^{G12D}and loss of *Ink4a/Arf* led to the identification and validation of a small panel of proteins that may predict PDAC development.[59] Kelly and coworkers used a similar GEMM to identify molecular markers by phage display that could be useful as targeted imaging agents. They identified and validated a potential biomarker named plectin-1 targeted peptide by conjugating it to magneto-fluorescent nanoparticles, which could be detected by intravital confocal microscopy and MRI.[18] While these results need validation in human samples, advantages of this preclinical strategy include the low heterogeneity of the genetic background, thus reducing the signal-to-noise ratio that often aggravates such proteomic approaches and the comparably easy validation approaches.

MOUSE MODELS OF PDAC FOR CHEMOPREVENTIVE AND THERAPEUTIC APPROACHES

Clinical development of cancer chemopreventive agents and strategies is an evolving field with unique features. Goals and end points may differ from therapeutic oncological agents. Whereas therapeutic oncological agent efficacy may be preliminarily assessed by surrogates such as reduction in tumour cell viability or of a tumour mass, few of such surrogates are available or easily addressable for cancer prevention end points. Thus, GEMMs are interesting candidates for chemopreventive approaches as such surrogates are more easily evaluable.

A better understanding of the molecular events and risk factors during development of PDAC may help to identify patients at increased risk, who may benefit from chemopreventive therapies. Potential risk situations include individuals with known heritable risk factors, those meeting the criteria for familial pancreatic cancer and patients with diagnosed cystic neoplasm such as IPMN and MCN prone to develop PDAC. While all of these scenarios present complex situations, GEMMs of familial pancreatic cancer[60–62] as well as those developing MCN and IPMN lesions progressing to PDAC have been described.[15 17 28 32] A GEMM with concomitant KRAS and EGFR activation showed development of the pancreatobiliary subtype of human IPMNs progressing to intraductal papillary mucinous carcinoma/PDAC,[28] suggesting potential usage of EGFR inhibitors in patients with IPMNs. Interestingly, a recent phase IIA study evaluating erlotinib in patients with IPMN not undergoing surgery described one patient having a complete clinical response.[63]

Besides being of value for elucidating molecular insights that may lead to clinically addressed hypotheses, GEMMs allow studying preventive and therapeutic agents at different stages of tumour development. Indeed, targeting distinct pathways and compartments may be only successful in certain stages of cancer development (figure 3). Successful chemopreventive approaches inhibiting PanIN and PDAC formation have been reported, including EGFR inhibition by gefitinib, Notch signalling inhibition using γ-secretase inhibitors and using a combination of aspirin and enalapril, an angiotensin-I-converting enzyme inhibitor.[33 64 65] Notably, inhibition of EGFR and Notch signalling led to an impressive reduction in PDAC development, suggesting that these cell fate regulating pathways may be a valuable approach for targeting preneoplastic lesions or early PDAC. Further chemopreventive approaches, which are summarised in two recent overviews, have been reported.[66 67] Notably, the success of GEMM for such chemopreventive strategies in terms of clinical relevance will likely depend on the applicability of results and the predictive value for clinically useful interventions and not so much on an exact recapitulation of all features of the respective human disease.

MOUSE MODELS OF PDAC FOR PRECLINICAL THERAPEUTIC STUDIES AND RESPONSE EVALUATION

The efficacy of a drug's antitumour activity is typically first established in preclinical *in vitro* studies. Among other limiting factors, the dynamic interactions between cancer cells and the microenvironment, a hallmark of PDAC, cannot be addressed. Thus, in vivo models are applied for

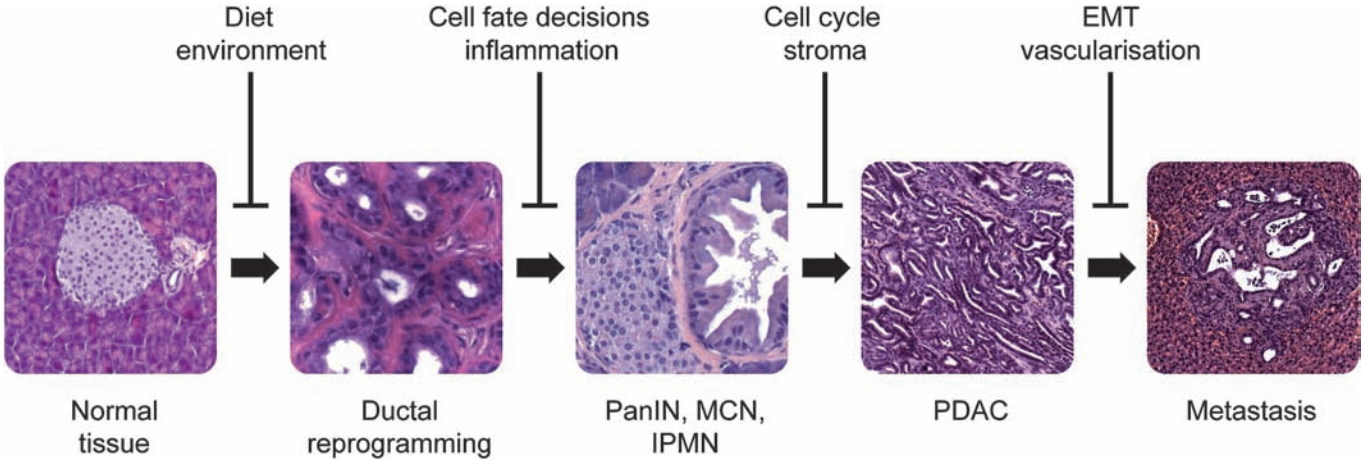

Figure 3 Chemopreventive and therapeutic strategies during pancreatic ductal adenocarcinoma (PDAC) development and progression. As targeting of distinct signalling pathways and cellular compartments may have different outcomes at different stages of PDAC development, genetically engineered mouse models (GEMMs) recapitulating the carcinogenic process are particularly suitable to test such interventions. Shown are proposed examples of interventional strategies at different stages of cancer development without intention of correctness. EMT, epithelial–mesenchymal transition; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; PanIN, pancreatic intraepithelial neoplasia.

evaluating a drug's clinical efficacy. While tumour measurements and response evaluation of subcutaneously transplanted tumours are simple and can be performed even manually, such non-invasive evaluation in orthotopic or endogenous models is somewhat more difficult and the tasks go far beyond tumour volume measurements. Since PDAC typically shows abundant desmoplasia and may spontaneously develop areas of necrosis over time, imaging techniques used for response evaluation should account for these tumour-inherent characteristics. Different modalities including high-resolution ultrasound and micro-CT have been used.[38 68] Multi-parametric magnetic resonance tomography and PET, while not yet reported in a preclinical GEMM study, may be promising

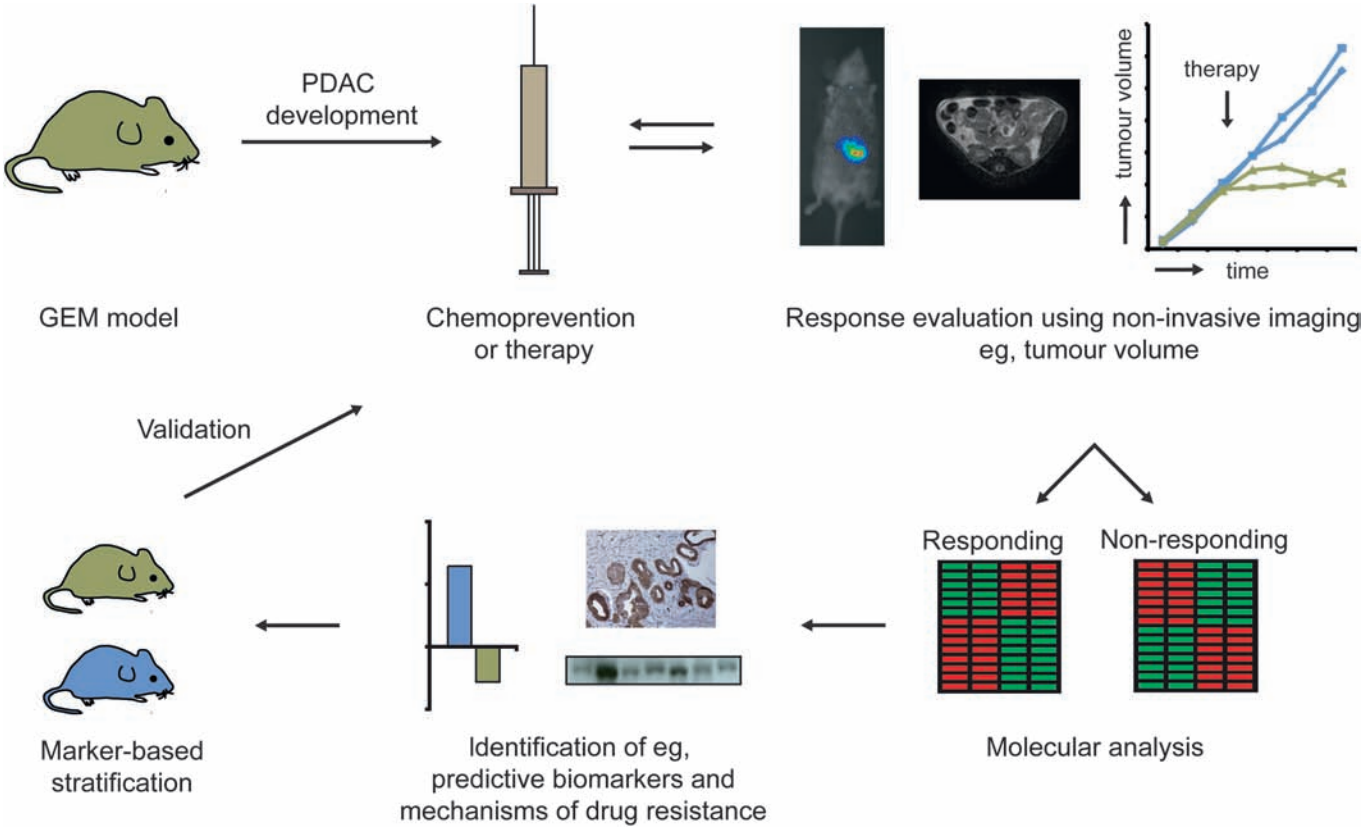

Figure 4 Potential utility of genetically engineered mouse models (GEMMs) for preclinical response evaluation and identification of biomarkers and resistance mechanisms. GEMMs undergoing preclinical therapeutic trials may be grouped by their response to therapeutic intervention and further molecularly classified. This may lead to identification of biomarkers for response prediction or mechanisms of drug resistance. These could then prospectively be validated in GEMM-based preclinical trials stratifying for potential markers. PDAC, pancreatic ductal adenocarcinoma.

modalities for biological imaging purposes (personal observation).

As our knowledge of the molecular alterations in PDAC and the amount of targeted therapies increases, testing of agents and combinations in accurate models may be one of the key factors in identifying successful therapeutic strategies. A problem that arises when xenografts are used for such approaches is that xenografts, while bearing typical molecular alterations of the respective human disease, typically do not reflect the intratumoural genetic heterogeneity that is a hallmark of PDAC. This may also be one of the reasons why cell-line-based identification of mechanisms of drug resistance has not been successful. Thus, GEMMs may be interesting candidates for evaluation of novel therapeutic strategies and for identification of predictive biomarkers and mechanisms of drug resistance (figure 4).

In a landmark study, Olive *et al* were the first to evaluate the preclinical treatment response in GEMM with endogenous PDAC.[38] They found that endogenous PDAC was poorly perfused and vascularised, highly different from subcutaneously transplanted tumours. Moreover, these PDACs were significantly less sensitive to gemcitabine treatment compared to the transplanted tumours. The authors went on to show that levels of gemcitabine and metabolites were lower in the endogenous PDAC, arguing for impaired drug delivery as an important mechanism of chemoresistance. By adding an Hh inhibitor to gemcitabine, the authors found an increase in intratumoural vascular density and gemcitabine concentrations, probably because of the stromal depletory effect of Hh inhibition. Clinical phase II studies are in progress to evaluate Hh inhibitors in PDAC.

Another key study investigated the utility of modern $Kras^{G12D}$-driven lung and pancreatic cancer GEMM to predict therapeutic response to standard chemotherapeutic and targeted therapies.[68] In an attempt to reproduce a clinical trial in a most similar way using sophisticated non-invasive imaging techniques, pharmacokinetic parameters and clinical end points, the authors found a high correlation of tumour responses in the GEMM with corresponding human clinical trials. These results suggest that GEMMs indeed are predictive for human tumour responses to treatment and can potentially be used for identification of predictive markers and response/resistance mechanisms.

CONCLUSIONS

Over the last decade, GEMMs of PDAC have become an invaluable tool for experimentally addressing tumour biological, microenvironmental and translational questions. Combining the original $Kras^{G12D}$-driven models with additional loss-of-function or gain-of-function alleles has helped in understanding the role of central genes and pathways during PDAC development. Lineage tracing approaches have begun to unravel the astonishing plasticity of different pancreatic lineages, their potential as cell of origin and the interaction with the microenvironment with its tumour-progressive and tumour-suppressive functions. Emerging fields of interest are approaches for early tumour detection, tumour responses and identification of biomarkers and mechanisms of drug resistance. Future challenges will likely include dissecting the contribution and interaction of individual signalling pathways in these processes and the identification of novel regulators. Preclinical diagnostic and therapeutic interventions using criteria employed in clinical trials will help establish the role of GEMMs regarding clinical relevance.

Acknowledgements We apologise to the many investigators for the inability to contain all references on this topic in this perspective.

Funding This work was supported by a grant from the German Federal Ministry of Education and Research (National Genomic Research Network (NGFN-Plus), 01GS08115 to JTS).

Competing interests None.

Patient consent Obtained.

Contributors P. Mazur: drafting of manuscript; J. Siveke: drafting, revision and final approval of manuscript.

Provenance and peer review Commissioned; externally peer reviewed.

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Genetically engineered mouse models of pancreatic cancer: unravelling tumour biology and progressing translational oncology

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Gut 2012 61: 1488-1500 originally published online August 26, 2011
doi: 10.1136/gutjnl-2011-300756